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Letter to the editor:

INTERVENTIONS ON SOY ISOFLAVONE MOLECULES TO IMPROVE THEIR THERAPEUTIC POTENTIAL FOR PROSTATE CANCER TREATMENT

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Recent global data suggest that prostate cancer represents the second most frequent cancer and the fifth leading cause of cancer deaths among men, with almost 1.4 million new cases and 375000 deaths in 2020 (Sung et al., 2021). Well-established risk factors for prostate cancer are family history, race and hereditary syndromes, while a limited number of modifiable risk factors may determine developing prostate cancer, so little evidence exists in terms of the disease prevention (Gandaglia et al., 2021). Soy isoflavones (genistein and daidzein are the best known representatives) are prominent as promising compounds in the prevention of prostate cancer, with observable but discrete effects (and some limitations) in cancer treatment, especially in its metastatic phase (Ajdžanović et al., 2019). In line with this, mechanistic studies indicate that soy isoflavones may affect various pathologically active signaling pathways in prostate cancer cells, downregulate the cancer cell androgen receptors, decrease the expression of prostate-specific antigen and matrix metalloproteinase, reverse prostate cancer cell epithelial to mesenchymal transition, contribute to epigenetic changes associated with the fate of cancer cells and suppress the angiogenesis that follows prostate cancer growth (Ajdžanović et al., 2019). The limiting factor for the clinical use of soy isoflavones is their low bioavailability, due to poor water solubility, rapid metabolism and excretion (Tang et al., 2019). Advances in chemo-, immuno- and radio-therapy dictate the fact that plant-derived compounds (formulated as dietary supplements/nutraceuticals) are not the first-line treatment for metastatic prostate cancer. However, soy isoflavone therapeutic ranking is rising as evidence accumulates regarding the effectiveness of combining therapeutic approaches with soy isoflavone participation (Ajdžanović et al., 2019). Given all the above, it can be said that there is a need for further tuning of prostate cancer treatment that involves soy isoflavones.

Enhancement of the therapeutic potency of soy isoflavones and upgrade of their pharmacokinetic profiles/bioavailability may be achieved by means of different interventions on these isoflavones, either by chemical engineering based on their structure or by designing efficient soy isoflavone delivery systems (Vodnik et al., 2021; Xiong et al., 2015). At the practical level, there are three modalities of such interventions: chemical modifications, synthesis of analogues and coupling with nanoparticles. Chemical modifications, including the possibility of increasing soy isoflavone molecules' lipophilicities through complexation with transient metal cations, to give modified compounds with desirable inputs on prostate cancer cell signaling machinery (Ajdžanović et al., 2015), still appear far from realization. Some practical experience is available with the other two modalities of interventions using soy isoflavone molecules, so their effects on prostate cancer cells and tumors is the main subject of elaboration in the following table (Table 1).

According to the data summarized in Table 1 (especially given the findings shown in bold), some progress has been made in improving the therapeutic potential of soy isoflavone molecules for the treatment of prostate cancer. At higher doses, genistein analogues have generally more pronounced antiproliferative effects on metastatic prostate cancer cells *in vitro* than daidzein, while thiogenistein shows even better performance than genistein within the same context. Nanosuspension of genistein, in combination with radiation, has been shown to be effective in suppressing prostate tumor growth. Genistein-gold nanoparticle conjugates possess lower toxicity than genistein against non-malignant human cells. Verification of these results requires further intensive research, primarily *in vivo* in animal models, and subsequently at the pre-clinical and clinical levels. Special attention should be paid to defining the optimal dosage range of modified soy isoflavone molecules in the treatment of prostate cancer, to achieve the best therapeutic results.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1: A review of interventions on soy isoflavone molecules that have been performed to improve their therapeutic potential for prostate cancer treatment

Molecule(s)/conjugates and mode of synthesis	Effects on prostate cancer cells and tumors	Reference
Copper(II) complexes (FPA-124 to FPA-127) of Schiff base derivatives of 3-formylchromone, the minimal biologically active structural motif of genistein	The effects were observed in PC-3 (human, androgen-independent) prostate cancer cell line. Metal complexes-induced cell growth inhibition was estimated according to IC_{50} (μM; the concentration effective in inhibiting viability of 50 % of cells measured by MTT cell proliferation assay). The effects were compared with the appropriate concentration of genistein. The copper complexes exhibited dose-dependent growth inhibitory effects in PC-3 prostate cancer cells. FPA-124 inhibited 50 % of PC-3 cell viability at a concentration of 10 μM (***the IC_{50} for genistein was 50 μM***). For FPA-125, the IC_{50} was 15 μM. FPA-126, in the context of PC-3 cells, reached the IC_{50} concentration at >50 μM, while IC_{50} for FPA-127 was 14 μM. FPA-124, FPA-125 and FPA-127 induced apoptosis in PC-3 prostate cancer cells, while FPA-124 showed the highest index of apoptotic PC-3 cells.	Barve et al., 2006
Genistein analogues:	The effects were observed in LNCaP (human, androgen-dependent) as well as in DU-145 and PC-3 (human, androgen-independent) prostate cancer cell lines, upon 3 days of exposure. An inhibition rate of >20 % was considered relevant to reflect cytotoxicity. The effect in a range of ±5 % was considered to be similar. Antiproliferative activity of genistein analogues was estimated according to IC_{50} (μM; the drug concentration effective in inhibiting 50 % of cell viability measured by WST-1 cell proliferation assay). All the effects were compared with the appropriate concentrations of genistein and daidzein.	Xiong et al., 2015
3-(4-Hydroxyphenyl)-4H-chromen-4-one, synthesized by Suzuki-Miyaura coupling reaction of 3-iodochromone with (4-hydroxyphenyl)boronic acid	Cytotoxic towards DU-145 cells at 50 and 100 μM (weaker than genistein, similar to daidzein), and towards PC-3 cells at 50 μM (weaker than genistein and daidzein). IC_{50} was much higher than IC_{50} of genistein and daidzein, regarding all three cell lines.	
3-Phenyl-4H-chromen-4-one, synthesized by Suzuki-Miyaura coupling reaction of 3-iodochromone with phenylboronic acid	Cytotoxic towards LNCaP cells at 50 and 100 μM (weaker than genistein and daidzein); towards DU-145 cells at 50 μM (weaker than genistein, similar to daidzein) and 100 μM (weaker than genistein, ***stronger than daidzein***), and towards PC-3 cells at 100 μM (weaker than genistein, similar to daidzein). IC_{50} was much higher than IC_{50} of genistein and daidzein, regarding all three cell lines.	

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3-(Pyridin-4-yl)-4H-chromen-4-one, synthesized by Suzuki-Miyaura coupling reaction of 3-iodochromone with pyridin-3-ylboronic acid	Cytotoxic towards LNCaP cells at 50 and 100 µM (weaker than genistein, similar to daidzein) and towards DU-145 cells at 100 µM (weaker than genistein and daidzein). IC_{50} was higher than IC_{50} of genistein and daidzein, regarding all three cell lines.	
3-(1-Isopropyl-1H-pyrazol-4-yl)-4H-chromen-4-one, synthesized by Suzuki-Miyaura coupling reaction of 3-iodochromone with 1-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole	Cytotoxic towards LNCaP cells at 50 µM (weaker than genistein and daidzein) and 100 µM (weaker than genistein, similar to daidzein); towards DU-145 cells at 100 µM (weaker than genistein, ***stronger than daidzein***), and towards PC-3 cells (weaker than genistein, ***stronger than daidzein***). IC_{50} was higher than IC_{50} of genistein and daidzein, regarding all three cell lines.	
3-(1-(sec-Butyl)-1H-pyrazol-4-yl)-4H-chromen-4-one, synthesized by Suzuki-Miyaura coupling reaction of 3-iodochromone with 1-sec-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole	Cytotoxic towards LNCaP cells at 50 and 100 µM (weaker than genistein and daidzein), towards DU-145 cells at 100 µM (weaker than genistein, similar to daidzein), and towards PC-3 cells at 100 µM (weaker than genistein, ***stronger than daidzein***). In the case of LNCaP cells, IC_{50} was higher than the values of genistein and daidzein; in the case of DU-145 cells, IC_{50} was higher than the IC_{50} of genistein, but similar to the IC_{50} of daidzein; and in the case of PC-3 cells IC_{50} was higher than the IC_{50} of genistein, ***but lower than the IC_{50} of daidzein***.	
3-(1-Isobutyl-1H-pyrazol-4-yl)-4H-chromen-4-one, synthesized by Suzuki-Miyaura coupling reaction of 3-iodochromone with 1-isobutyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole	Cytotoxic towards LNCaP cells at 50 µM (weaker than genistein and daidzein) and 100 µM (***stronger than genistein and daidzein***); towards DU-145 cells at 100 µM (weaker than genistein, ***stronger than daidzein***), and towards PC-3 cells at 100 µM (weaker than genistein, ***stronger than daidzein***). IC_{50} was higher than the IC_{50} of genistein and daidzein, regarding all three cell lines.	
3-(1-(Pentan-2-yl)-1H-pyrazol-4-yl)-4H-chromen-4-one, synthesized by Suzuki-Miyaura coupling reaction of 3-iodochromone with 1-	Cytotoxic towards LNCaP cells at 50 and 100 µM (weaker than genistein and daidzein), towards DU-145 cells at 100 µM (weaker than genistein, ***stronger than daidzein***), as well as towards PC-3 cells at 50 µM (weaker than genistein, ***stronger than daidzein***) and 100 µM (similar to genistein, ***stronger than daidzein***). For all three cell lines, IC_{50} was higher than the IC_{50} of genistein, ***but lower than the IC_{50} of daidzein***.	

(pentan-2-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole **3-(1-(Pentan-3-yl)-1H-pyrazol-4-yl)-4H-chromen-4-one**, synthesized by Suzuki-Miyaura coupling reaction of 3-iodochromone with 1-(pentan-3-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole	Cytotoxic towards LNCaP cells at 50 µM (weaker than genistein and daidzein) and 100 µM (weaker than genistein, similar to daidzein); towards DU-145 at 100 µM (weaker than genistein, ***stronger than daidzein***), and towards PC-3 at 100 µM (similar to genistein, ***stronger than daidzein***). In the case of LNCaP cells IC_{50} was ***lower than IC_{50} of genistein and daidzein***; in the case of DU-145 cells IC_{50} was higher than the IC_{50} of genistein, ***but lower than the IC_{50} of daidzein***, and in the case of PC-3 cells IC_{50} was higher than IC_{50}s of genistein and daidzein.	
Thiolated genistein analogue – **thiogenistein** chemically attached to gold surface as a self-assembled monolayer	The effects were observed in DU-145 (human, androgen-independent prostate cancer) cells and PNT2 (normal prostate epithelium) cells, upon exposure to genistein and thiogenistein in six concentrations (200, 100, 50, 25, 12.5 and 6.25 µM) for 6, 24 and 72 h. Cytotoxicity of the treatments, as well as cell viability and morphology following their application were determined. Thiogenistein in a concentration of 50 µM reduced the viability of DU-145 prostate cancer cells to 89.49 % and 66.12 % after 6 and 24 h of incubation, respectively (***faster than the corresponding genistein***). Viability of DU-145 cells was reduced to 60.8 % after 6 h of exposure to 100 µM of thiogenistein (***a stronger effect than that provided by the corresponding genistein***). The cytotoxicity for the three highest concentrations of thiogenistein increased over time and reached 84 % after 72 h (***a stronger effect than the corresponding genistein concentrations provided***). After 6 h of incubation with 100 µM thiogenistein, DU-145 prostate cancer cells ***became more rounded in contrast to genistein-treated cells which retained their elongated shape***. Thiogenistein, in a concentration of 50 µM, decreased the viability of normal prostate PNT2 cells to 79.88 %, after 72 h of incubation (***much less than the corresponding genistein***). The cytotoxicity of 50 µM thiogenistein against PNT2 cells was 19.07 % (***lower than that provided by the corresponding genistein***). In normal prostate epithelium PNT2 cells, after 72 h of incubation with 50 µM thiogenistein, morphology and proliferation were not changed (***the loss of shape and arrested proliferation were observed upon the corresponding genistein treatment***).	Stolarczyk et al., 2021

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BIO 300 - nanosuspension of synthetic genistein	Young adult immunocompromised nude mice were used to evaluate the effect of BIO 300 on prostate tumor xenograft growth. PC-3 or LNCaP human metastatic prostate cancer cells were injected subcutaneously into the mice. BIO 300 was administered daily, at a dose of 200 mg/kg by oral gavage, alone (starting 3 days before sham radiation therapy) or starting 3 days before radiation therapy (prophylactic) or 2 h after radiation therapy, until euthanasia. In hormone-independent PC-3 tumor-bearing mice, tumor growth inhibition on day 18 was 85.75 % for BIO 300 alone, 99.27 % for BIO 300 administered starting 3 days before radiation therapy, and 90.13 % for BIO 300 administered starting 2 h after radiation therapy. Animals treated with radiation therapy and BIO 300 starting prophylactically or 2 h after radiation therapy demonstrated minimum (20 %) morbidity. In hormone-dependent LNCaP tumor-bearing mice, tumor growth inhibition on day 18 was 60.82 % for BIO 300 alone, 98.69 % for BIO 300 administered starting 3 days before radiation therapy, and 99.69 % when BIO 300 was administered starting 2h after radiation therapy. Until 44 days after radiation therapy, there was no morbidity in mice that received BIO 300 starting 3 days before radiation therapy and in mice that received BIO 300 starting 2 h after radiation therapy.	Jackson et al., 2019
Genistein-gold nanoparticle conjugates **Gen@AuNPs1** (d_{av} = 10 ± 2 nm; ~46 % of genistein loading) and **Gen@AuNPs2** (d_{av} = 23 ± 3 nm; ~48 % of genistein loading), synthesized by an environmentally friendly method, using a dual role of genistein to reduce Au^{3+} and stabilize the formed AuNPs, with no additional component	The antiproliferative activities of Gen@AuNPs (1-50 µg/mL for 72 h) were evaluated using the MTT cell proliferation assay *in vitro* on three metastatic prostate cancer cell lines (PC-3, DU-145 – human, androgen-independent; and LNCaP – human, androgen-dependent). Gen@AuNPs-induced cell growth inhibition was estimated according to IC_{50} (µM). The effects were compared with the appropriate concentration of genistein. Gen@AuNPs1 inhibited prostate cancer cell viability more prominently than Gen@AuNPs2 in all examined cell lines. Namely, for Gen@AuNPs1, IC_{50} values (µg/mL) were: 19.6 (LNCaP), 39.6 (DU-145) and 22.6 (PC-3). For Gen@AuNPs2 IC_{50} values (µg/mL) were: 29.3 (LNCaP), >50 (DU-145) and 46.3 (PC-3). As seen, PC-3 cells were moderately more sensitive to Gen@AuNPs then DU-145. However, for free genistein IC_{50} values (µg/mL) were lower: 13.9 (LNCaP), 21.0 (DU-145) and 22.3 (PC-3). Incubation of PC-3 cells with free genistein or Gen@AuNPs1 did not result in a significant apoptosis or affect autophagy, while the cell proliferation was significantly inhibited. Both Gen@AuNPs maintained low toxicity (***lower than genistein***) against non-malignant human lung fibroblast MRC-5 cells.	Vodnik et al., 2021

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